PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:
C12N 15/10, C12Q 1/68	A1	WO 91/05861
		(43) International Publication Date: 2 May 1991 (02.05.91)

(21) International Application Number: PCT/US90/05938

(22) International Filing Date: 16 October 1990 (16.10.90)

(30) Priority data:
422,392 16 October 1989 (16.10.89) US

(71) Applicant: GENELABS INCORPORATED [US/US]; 505 Penobscot Drive, Redwood City, CA 94063 (US).

(72) Inventors: LOVETT, Michael ; 416 Durant Way, Mill Valley, CA 94941 (US). REYES, Gregory, R. ; 2112 St. Francis Drive, Palo Alto, CA 94303 (US). WEISSMAN, Sherman, M. ; 495 St. Ronan Street, New Haven, CT 06511 (US). JORGENSON, Linda, M. ; 555 Addison Avenue, Palo Alto, CA 94301 (US).

(74) Agent: DEHLINGER, Peter, J.; 350 Cambridge Avenue, Suite 100, Palo Alto, CA 94306 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.
With amended claims.

Date of publication of the amended claims:
10 June 1993 (10.06.93)

(54) Title: NON-SPECIFIC DNA AMPLIFICATION

(57) Abstract

A method of non-specifically amplifying different-sequence fragments in a mixture of duplex DNA fragments is disclosed. The fragments are provided with end linkers, and the mixture is amplified by successive primer-initiated replication. Also disclosed is a method of cloning cDNA species which are homologous to a region of contiguous genomic DNA and are selected from a mixture of cDNA species.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SK	Slovak Republic
CI	Côte d'Ivoire	LI	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	MC	Monaco	TG	Togo
DE	Germany	MG	Madagascar	UA	Ukraine
DK	Denmark	ML	Mali	US	United States of America
ES	Spain	MN	Mongolia	VN	Viet Nam
FI	Finland				

AMENDED CLAIMS

[received by the International Bureau on 22 April 1991 (22.04.91);
original claims 2 and 3 cancelled;
original claims 1 and 4-15 renumbered as claims 1 and 2-13
wherein claims 1, 11 and 14 are amended;
claim 16 amended and renumbered as claim 15 (5 pages)]

1. A method of isolating from a mixture of cDNA fragments two or more specific cDNA fragments which are homologous to a contiguous region of genomic DNA, comprising

obtaining the contiguous region of genomic DNA,

preparing the mixture of cDNA fragments to contain end-terminal priming sequences,

denaturing the fragments of the mixture to produce single fragment strands with end-terminal priming sequences,

isolating from said mixture of fragments in single-strand form, those single fragment strands which hybridize to the contiguous region of genomic DNA,

hybridizing the single fragment strands which are homologous to the genomic region with a primer whose sequence is complementary to said end-terminal priming sequences on each fragment strand, to form strand/primer complexes,

converting the strand/primer complexes to double-strand fragments in the presence of polymerase and deoxynucleotides,

denaturing the double-strand fragments, and

repeating said hybridizing, converting, and denaturing steps until a desired degree of cDNA amplification is achieved.

2. The method of claim 1, wherein the contiguous genomic DNA section is bound to a solid support.

3. The method of claim 1, wherein the contiguous genomic DNA section is contained in yeast artificial chromosomes.

4. The method of claim 1, wherein the contiguous genomic DNA section represents the bovine leukosis virus genome and the mixture of cDNA species is a cDNA library made from mRNA obtained from a cell line infected with a virus derived from cell line 10C9.

5. The method of claim 1, for use in identifying cDNAs which correspond to genes located in the same chromosomal region as a known gene, wherein said genomic fragments are obtained by

(a) preparative size fractionating of genomic DNA fragments containing the known gene,

(b) ligating linkers to the genomic DNA fragments, which are useful as primers for sequence-independent amplification, to the ends of the DNA fragments and digesting with a restriction enzyme to eliminate the presence of redundant linkers on the ends of the DNA fragments,

(c) mixing the DNA fragments with DNA polymerase, all four deoxyribonucleotides, and primers homologous to the linkers present on the ends of the DNA fragments, and

(d) reacting the mixture under conditions to produce sequence-independent amplification of the DNA fragments.

6. The method of claim 5, wherein the preparative size fractionating of genomic DNA fragments containing the known gene involves identification of the DNA fragments of interest by adding primers homologous to the known gene, DNA polymerase, and all four deoxyribonucleotides, to the gel matrix and treating the gel matrix under conditions which promote amplification of the region of the known gene defined by the primers.

7. The method of claim 1, wherein the known gene is a growth factor or growth factor receptor gene.

8. The method of claim 7, wherein the known gene is an interleukin.

9. The method of claim 8, wherein the known gene is IL-5 and the mixture of cDNA species is a T-cell cDNA library.

10. The method of claim 7, wherein the known gene is erythropoietin and the mixture of cDNA species is a renal cDNA library.

11. The method of claim 1, wherein said preparing of cDNA species to contain end-terminal priming sequences includes

attaching a double-strand linker to the fragments of the cDNA mixture,

denaturing the fragments to produce single fragment strands with linker-strand ends, where the linker-strand ends serve as end-terminal priming sequences,

hybridizing the single strands with a primer whose sequence is complementary to a linker-strand end on each fragment strand, to form strand/primer complexes,

converting the strand/primer complexes to double-strand fragments in the presence of polymerase and deoxynucleotides, and

repeating said denaturing, hybridizing, and converting steps until a desired degree of amplification is achieved.

12. The method of claim 1, wherein said preparing of cDNA species to contain end-terminal priming sequences includes the cloning of the cDNA species into a suitable vector and using the known 5′ and 3′ vector sequences, which flank the cDNA insert, as end-terminal priming sequences.

13. The method of claim 12, wherein said preparing further includes the amplification of the cDNA species by mixing the cDNA species containing said end-terminal priming sequences with DNA polymerase, all four deoxyribonucleotide triphosphates, and primers homologous to the cDNA end-terminal priming sequences, and reacting the mixture under conditions to produce sequence-independent amplification of the single-stranded cDNA species.

14. The method of claim 1, which further includes cloning the amplified specific cDNAs into a vector.

15. A method of cloning two or more cDNA species which are homologous to a contiguous region of genomic DNA and are selected from a mixture of cDNA species, said method comprising,

(a) preparing cDNA species to contain end-terminal priming sequences,

(b) isolating single-stranded cDNA species on the basis of their hybridization to a first set of selected genomic fragments,

(c) mixing the isolated single-stranded cDNA species with DNA polymerase, all four deoxyribonucleotide triphosphates, and primers homologous to the cDNA end-terminal priming sequences,

(d) reacting the mixture under conditions to produce sequence-independent amplification of the single-stranded cDNA species,

(e) isolating single-stranded cDNA species, from the mixture of step (d), on the basis of their hybridization to a second set of selected genomic fragments,

(f) mixing the isolated single-stranded cDNA species of step (e) with DNA polymerase, all four deoxyribonucleotide triphosphates, and primers homologous to the cDNA end-terminal priming sequences,

(g) reacting the mixture under conditions to produce sequence-independent amplification of the single-stranded cDNA species,

(h) cloning the amplified cDNA species into a vector.